U.S. Department of Agriculture Agricultural Research Service

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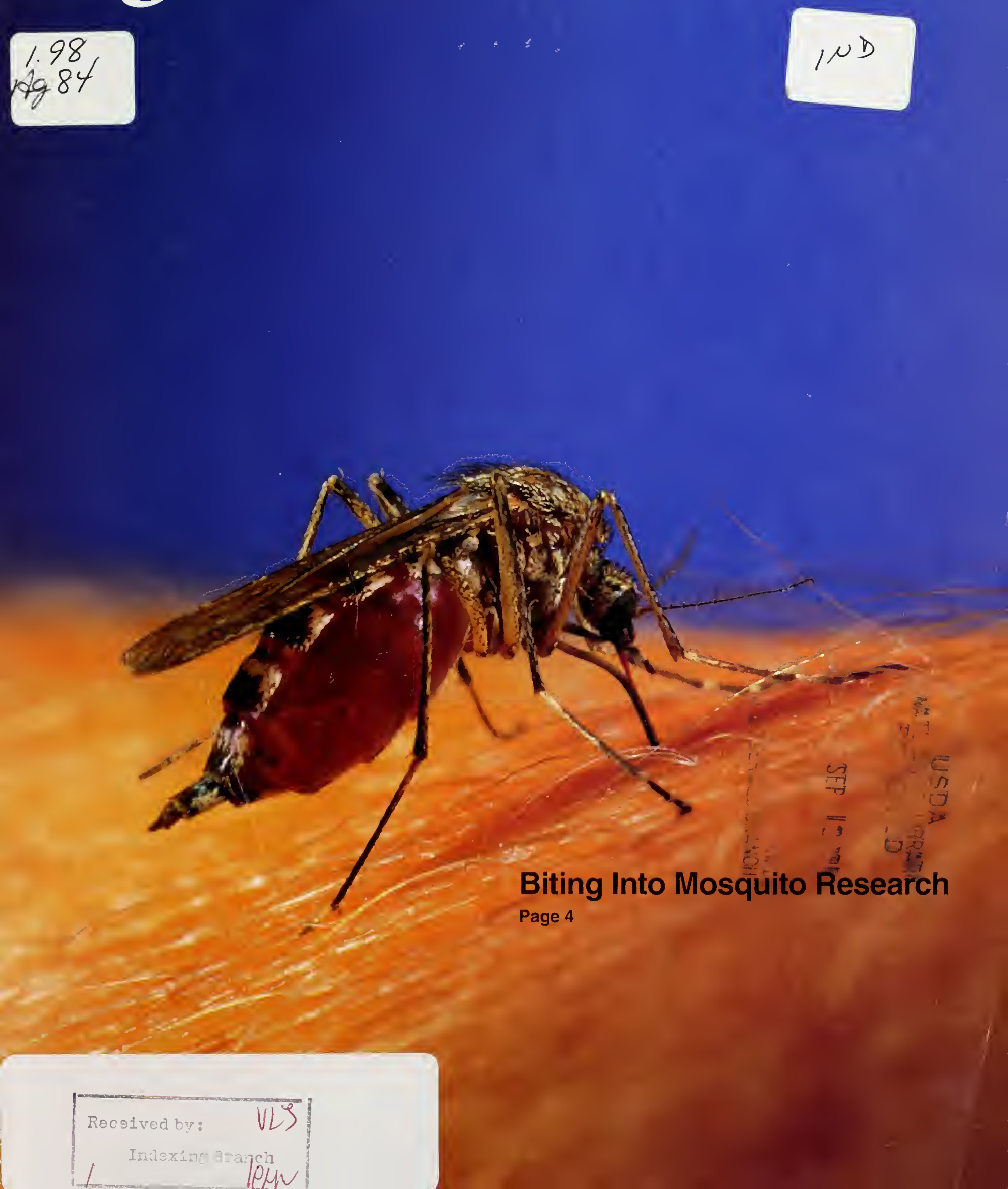
# Agricultural Research





## Biting Into Mosquito Research

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## Oh No, Mosquitoes!

It's probably fair to say that when we donate blood, most of us would like to control when we donate it—and to whom.

So it's maddening when, during a leisurely chat with the neighbors on a warm summer night, you feel what you think is an ordinary itch on your ankle and you look down to see: A BLOOD-SUCKING MOSQUITO. You squash her, but it's too late; she's already bitten. And within minutes, her legacy appears on your ankle: a dime-sized welt that may itch all night.

This is how thousands of Americans experience mosquitoes. But for our military troops stationed abroad, as well as for our friends in neighboring countries, a mosquito bite could mean debilitating, perhaps even deadly disease.

Malaria, credited with causing more U.S. military casualties in some theaters of operation in World War II than the war itself, causes enlargement of the liver and spleen, fever, and—following each malarial attack—depression and inability to concentrate. Caused by microorganisms carried by the *Anopheles* species, malaria sometimes leads to death.

Another mosquito, *Aedes aegypti,* most commonly spreads yellow fever, which may result in fever, headache, dizziness, muscular pain, nausea and vomiting, jaundice, collapse, and even death.

Although yellow fever and malaria have been eradicated in the United States, the fact that the transmitters of these diseases are still here concerns officials.

*Aedes, Anopheles,* and *Culex* species transmit filarial worms, which can cause fever, chills, and swelling of the lymph nodes. Infection can mean gross enlargement of legs, arms, or other body parts—called elephantiasis. Filarial worms are not a concern to those of us in the continental United States, but they are for military personnel stationed abroad. And they are a threat to dogs in this country; the heartworm is a filarial worm transmitted in much of the United States by mosquitoes.

The same three mosquito species—along with six others—also spread viruses causing encephalitis, inflammation of the brain that can kill, and have done so in the United States in the last few years.

*Ae. aegypti* is the main transmitter of dengue fever, whose victims experience mottling of the skin, rash, bleeding, backache, muscle or joint pain, and severe headaches and weakness. Like malaria and yellow fever, this disease is currently a problem only for American troops and residents in other countries. But the fact that its vector flourishes in the United States makes dengue fever a potential health threat here, too.

Meanwhile, all of these species and others—more than 150 in all—span the country, terrorizing the evening barbecue. Even Alaska has serious mosquito problems. And folks in the southern states swat them during most months of the year.

Because it's many species doing the dirty work—and each species has a slightly different life cycle, behavior pattern, and preferred blood meal host—the picture gets even more complicated. So ARS is going after this nasty biting pest with almost every weapon in the insect control arsenal.

Scientists are using biological controls, repellants, trapping, computer modeling, and genetic engineering to make mosquitoes less troublesome. They look at the human victims ourselves—how to clothe and shelter us so mosquitoes can't get to us and even how to mask our body chemistry so that mosquitoes can't find us.

Itching to learn more? Turn to "Slapping the Mosquito—Scientifically."

**Jessica Morrison Silva**
Agricultural Research Service

Agricultural Research

Cover: Mosquito research at the ARS Medical and Veterinary Entomology Research Laboratory in Gainesville, Florida. (K-4188-1)

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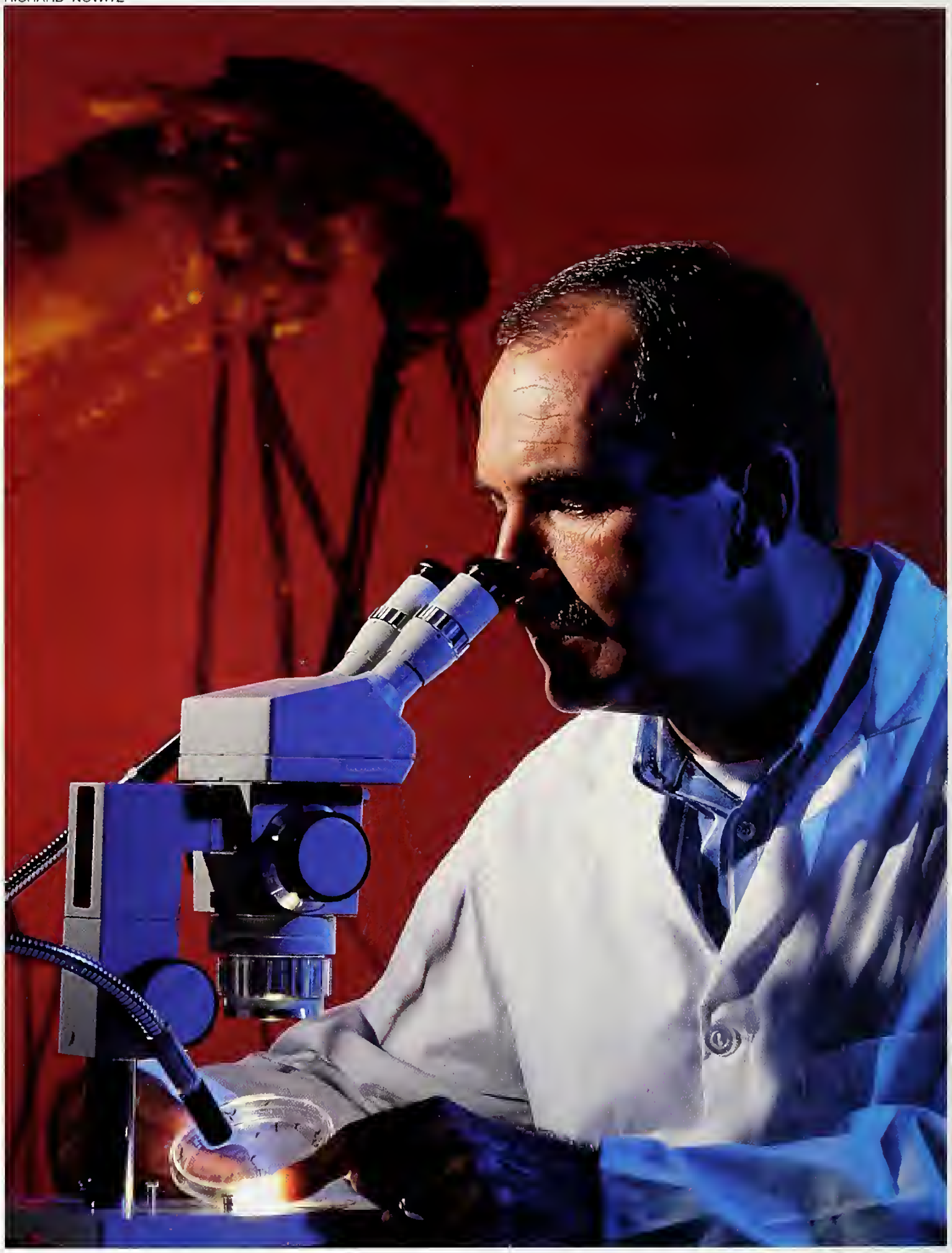
# Slapping the Mosquito—Scientifically

## Wait—before you roll up this magazine, check out the latest anti-skeeter strategies!

RICHARD NOWITZ



**Donald Barnard examines yellowfever mosquitoes to see if they have had their blood "meal," a prerequisite for complete egg development. (K-4159-5)**

It's too bad the mosquito is such a pain (not to mention itch)—its life cycle is really quite fascinating.

Just listen to Gary A. Mount, Donald L. Barnard, and Jack A. Seawright, of ARS' Medical and Veterinary Entomology Research Laboratory in Gainesville, Florida, and you may even be tempted to admire the delicate little horrors.

For all larvae, life begins in still water. For some species, this means water trapped in discarded tires, buckets, planters, ditches, tree holes, and other containers. Other species hatch in lakes or even sewage ponds.

The larvae (one-tenth to one-fourth inch long) usually float at the top of the water, breathing through a snorkel-like device. They stay afloat because they need air to survive. "If something upsets them, they go down quickly, stay down a minute or so, and then come back up," Barnard says.

A brushlike apparatus circulates water containing food particles through the larva's mouth. Mosquito larvae are filter feeders, feeding on anything in the water that they can filter through their mouths, such as organic debris and small invertebrates.

Barnard says organic matter is plentiful wherever mosquitoes are because the water they live in is stable. Decomposing vegetation usually sinks down into standing water, and because food for algae, bacteria, fungi, and invertebrates thrives. "It's like a community of organisms within the container."

Adults are less alike, in behavioral terms, than larvae. Some adults breed and live near fresh water, while others prefer brackish. Some choose foul water; others, clear. Some breed in anything that will hold water while others stick to just tree holes. Although most species become active and bite at dusk, some also attack during the day.

Many species prefer to bite birds and mammals, but others are known to attack reptiles, amphibians, fish, and even other insects for a blood meal.

Actually, blood isn't a source of sustenance; both male and female mosquitoes seek plant nectar and juices

for that. Females use blood protein to complete egg development before they lay their eggs.

Entomologists Albert Undeen and James J. Becnel at the Gainesville lab study natural mosquito control. One of the most promising organisms right now, Undeen says, is a tiny parasite called *Edhazardia aedis*. Its spore looks like a tiny egg and works like a hypodermic syringe to infect mosquito larvae, attacking container-breeding mosquitoes like the yellow and dengue fever transmitter *Aedes aegypti*.

As they are filter feeding, explains Becnel, mosquito larvae ingest dormant spores of the parasite. When the spores germinate, they put out a tubelike projection that penetrates the larva's gut and infects it.

Some larvae die, but most emerge as infected adults. Inside an infected adult female, the parasite produces yet another kind of spore that infects the eggs.

Many larvae that emerge from infected eggs die. Their corpses release the original type of spore that, in turn, infects other larvae, completing the parasite's life cycle.

Females that emerge from infected eggs and somehow survive also lay infected eggs.

"This transmission from females to offspring is what makes this such a good candidate for biological control of container-breeding mosquitoes," Undeen says.

Becnel explains, "With container breeders, you must have a pathogen that the mosquito itself carries around because you can't find all the other little containers." With *E. aedis,* female mosquitoes take care of spreading the disease.

In laboratory tests, the parasite infects and kills 100 percent of larvae in a container—depending on the dosage of spores used. "But that doesn't mean we're going to get that kind of control in the field, generation after generation," Undeen warns. Small-scale field tests may begin soon, if the Environmental Protection Agency approves them.

As Becnel prepares for field tests on *E. aedis,* Undeen is steering his own research in new directions. He plans to search for spots where "you'd expect to find mosquitoes in large numbers but you don't." Previously, scientists brought mosquitoes into the lab and looked for diseases in them. This could have been biasing the search toward chronic pathogens that would debilitate mosquitoes but perhaps not actually kill them, Undeen says.

He will begin looking in waste water habitats this year, following populations closely and trying, ironically, to not find mosquitoes. If an area that should have mosquitoes proves to have none, Undeen will look for organisms that could be keeping the region mosquito-free. That organism could be, Undeen reasons, a powerful biocontrol agent. "I may be going out on a limb with this approach, but it's worth a try."

## Protecting the Victim

Gainesville scientists have invested three decades of research in protecting people from biting insects—outdoor workers and recreationists here and military personnel abroad.

"The research emphasis has been on malaria transmitters," Barnard says, pointing out that the Department of Defense funded much of this research.

The work has led to development and wide use of deet, a strong repellant of biting flies, mosquitoes, ticks, and chigger mites. deet was discovered by ARS chemists in Beltsville, Maryland, and entomologists of the Gainesville lab nearly 40 years ago. It has been used both by civilians and troops ever since.

But deet is coming under fire. Its oiliness makes it messy to use. It can occasionally irritate skin and will burn mucous membranes and sensitive areas, for example around the eyes and mouth. And it dissolves some paints and plastics such as vinyl.

So a few years ago, 3M Company made new deet formulations and entomologist Carl E. Schreck tested them at Gainesville, recommending which one might be best suited for military use. The result: a new cream with special polymers that help it stick to the skin better. "The new formulation has half of the active ingredient, with just 35 percent deet but performs just as well as the military formulation that had 75 percent."

Deet strongly repels species of the *Aedes* and *Culex* genera but isn't as effective with other important genera, like *Anopheles*.

Schreck says that the program to develop new repellants never quits, because "we're always trying to find the ideal product that both repels all mosquitoes and doesn't irritate even sensitive skin."

Over the last three decades, chemists Albert B. DeMilo and the late Terrance McGovern of the Insect Chemical Ecology Laboratory at Beltsville, Maryland, developed 600 to 800 new materials for Schreck to

RICHARD NOWITZ

**Mosquito larvae infected with the protozoan *Edhazardia aedis*. The males will die; females will lay protozoan-spiked eggs. (K-4157-1)**

**Entomologist Carl Schreck examines a collection tube containing yellowfever mosquitoes *(Aedes aegypti).* (K-4161-1)**

screen for repellancy. That work has led to a few good candidates; all are, unfortunately, more expensive to develop and use than simply staying with deet.

"Industry is reluctant to spend the development moneys it takes to get EPA registration, because it's not a very economical investment for them," Schreck says. A company could invest several million dollars and "find they only sell the product in May, June, and July, when mosquitoes are out in full force. Then they'd have to wait until next season."

"Basically, the synthesis programs that industry used to run are at about zero today. It's up to us to develop these things," Schreck says.

Schreck has also extensively tested a mosquito killer developed by chemists in Britain. It is a synthetic pyrethroid called permethrin.

In recent studies, Schreck found that permethrin lasts 6 to 9 months on tents—in terms of both knocking down the total number of mosquitoes entering a tent and reducing the number of bites. "For up to 9 months, people got either zero bites or very few," he says.

The tents were kept outside for a year straight—a situation that probably wouldn't occur for the average camper or even military personnel. "That should give you some idea of how effective the treatment is. In intermittent use, one treatment would probably last the tent's life," he says.

The compound is approved for spray application to civilian clothing. It also has EPA registration for U.S. armed forces use in several forms for application to clothing. In fact, permethrin, along with the new formulation of deet, was widely used by troops in the Persian Gulf during Operation Desert Storm.

### Go Bite Somebody Else

Schreck has also begun a project for personal protection—one with an interesting twist. Instead of focusing on victims' clothing or tents, he's looking at the victims themselves. He's trying to answer that age-old question: Why do mosquitoes abso-

## Bioengineering a Better Skeeter

Is any mosquito good?

Possibly, if you live in or are visiting a country where malaria is endemic and you're bitten by one that doesn't transmit malaria—instead of by one that does.

Geneticist Andrew Cockburn has developed a genetic engineering technique that he hopes to one day use to create malaria-resistant mosquitoes. Tiny needles, new genes, and insect eggs are spun in an ordinary lab vortex in saline solution. The needles gently pierce the eggs, allowing new material to enter. Cockburn has transferred either a test gene or dye into the eggs of house flies, Caribbean fruit flies, stable flies, and *Drosophila* fruit flies. [See *Agricultural Research,* February 1990, p. 16-17]

As yet, Cockburn's technique has not been able to penetrate the tough eggs of the mosquito. But he is confident that by fiddling with vortex speed and needle number, he can slip new material into those, as well.

In case (and in hopes) the technique does create malaria-resistant mosquitoes, Cockburn and colleagues are also looking for quick ways to separate those from normal mosquitoes.

One possibility is to locate the gene that gives DDT-resistant mosquitoes that trait and attach it to the gene for malaria resistance. Then, after using the spin and pierce technique, scientists could theoretically select which eggs had taken up malaria resistance by dosing the whole group with DDT. If a mosquito dies from DDT, then it doesn't have either gene. But if it survives the noxious chemical, then it has both new genes and should be bred for release.

If the team succeeds in creating malaria-resistant mosquitoes, officials in areas with malaria could kill off a population with insecticides. Then, rather than letting it build back up naturally, officials would replace the insects with Cockburn's malaria-resistant strain. Resistant mosquitoes would pass the trait along to future generations, maintaining the malaria-carrying potential of the population at a lower level.

"You'd still have biting mosquitoes," he says, "but you wouldn't get malaria."—**Jessica Morrison Silva**

lutely adore you, while someone standing next to you gets relatively few bites?

Schreck tested volunteers in the lab to see who most and least attracted hungry mosquitoes.

Volunteers put a hand into a chamber. A fan blew air over the hand and into a chamber next door, which housed 100 female mosquitoes. Schreck counted how many responded.

The most attractive: technician Daniel Smith.

To find out what makes the difference, Schreck had Smith roll a few glass marbles in his hands and drop them into ethanol. The chemical removed the residue so that chemist David C. Carlson could analyze it in a gas chromatograph.

Results are still being analyzed, and studies continue. The trail could lead scientists to what mosquitoes are responding to when they search for a blood meal and then allow the scientists to find a chemical that will mask that component.

Schreck says they will even consider whether dietary factors affect the insect's attraction to different people.

### Cow Breath Comes in Handy

Octenol, a component of the breath of ruminants, may offer a unique new mosquito control method. Originally studied in Africa by scientists trying to control tsetse flies, octenol has proven to be very attractive to mosquitoes, says entomologist Daniel Kline.

In outdoor cage tests, octenol attracted the same number of mosquitoes as carbon dioxide, another proven attractant. But combining the two produced a synergistic reaction: 3 to 10 times more mosquitoes were attracted than with either compound alone. Scientists believe the octenol attracts mosquitoes from far away, while carbon dioxide attracts closer ones.

The best way to increase a mosquito catch is to combine the two, Kline says. And so the researchers are "taking a monitoring tool and adapting it to make it a control device."

He uses a standard mosquito trap with a wick that emits octenol. "We're trying to figure out how much octenol needs to be released for ideal results. With a wick, the release rate is subject to wind and temperature, and we'd like to be a little more precise than that."

He also says he has to improve the trap so it can be produced more cheaply if it is to be used as a control method.

Finally, the method needs to be proven in large-scale field tests. Within the next 5 years, Kline hopes to go into the Florida Everglades, mark mosquitoes with a fluorescent dye, and test the system with natural populations.

Until then, he'll continue to work with two Florida mosquito districts in small-scale tests to find out how many

RICHARD NOWITZ

**Yellowfever mosquitoes *(Aedes aegypti)* attack the untreated arm of researcher Ken Posey. Below, an arm that is treated with the repellant deet suffers far fewer bites. (K-4158-7,18, 19)**

RICHARD NOWITZ

Biological technician Ken Posey clocks length of time mosquitos are repelled by various treatments. (K-4157-16)

traps are needed, how far apart to put them, what release rate for carbon dioxide and octenol is best, and whether mosquitoes respond throughout their lives or only during certain periods.

But if Kline could prove the method effective, mosquito abatement officials and homeowners alike would have a nature-friendly mosquito control method.

This project has so far centered on the salt marsh mosquito, *Aedes taeniorhynchus,* a terrible biting pest that occurs as far north as New Jersey. This is a wetland breeder, and "wetlands are very sensitive as far as using chemical pesticides," Kline says. So a removal trapping method would be welcomed, because it would offer an alternative to the chemical pesticides that are currently used.

Though he developed the system with the salt marsh mosquito, Kline says that octenol attracts 4 other species of the 69 he tested, including 3 that cause disease.

*Psorophora columbiae* is a freshwater flood mosquito associated with rice fields and suspected of spreading Venezuelan equine encephalitis in the United States every few decades.

*Mansonia titillans* and *Coquillettidia perturbans,* two biting pests implicated in transmitting eastern equine encephalitis, are also attracted.

Kline says octenol could replace carbon dioxide in traps used to monitor any of these species. Abatement officials often have trouble getting or using carbon dioxide, which comes in the form of dry ice or in compressed gas cylinders. A small vial or wick of octenol would be a lot more efficient and would provide a "good index of the mosquito numbers that are out there," he says.

In fact, the Collier County Mosquito Control District in Florida is already using octenol to monitor for salt marsh mosquitoes and has reported very good results.

### Mosquito Population Prediction

Engineer Danel G. Haile and entomologist Dana A. Focks have developed three computer simulation models for mosquitoes.

MALSIM predicts how many cases of malaria military officials can expect with certain weather patterns and control measures. The scientists furnished the program with basic information about the mosquito's life cycle, including how temperature, moisture, and other climatic factors affect its survival. They told the program that malaria must incubate inside a mosquito 12 to 14 days before it can be transmitted to another victim.

The model predicts the benefits of different mosquito control programs, including aerial spraying or personal protection measures for troops.

Though the model hasn't been corroborated fully in a field situation, the military is already using it as a training tool for new recruits. The program is first run to demonstrate how many of them will get malaria if they don't use their personal protection gear.

The program is run a second time, with personal protection gear in place. The simulations serve as good motivation to use permethrin on clothes and deet on skin.

PCSIM simulates population dynamics of *Psophora columbiae,* the riceland mosquito, while CIMSIM models container-inhabiting mosquitoes. Like MALSIM, these programs predict insect numbers based on weather conditions and control measures.

Once validated, computer simulations could be used to develop integrated management strategies for mosquito abatement programs.—By **Jessica Morrison Silva,** ARS.

*To contact ARS scientists mentioned in this article, write or call the Editor, Agricultural Research Magazine, Bldg. 005, 10300 Baltimore Ave., Beltsville, MD 20705-2350. Phone (301) 344-3280.* ◆

RICHARD NOWITZ

These white marbles could help explain why mosquitoes seem to prefer certain individuals. Subjects who are particularly "tasty" roll the marbles in their hands. Residues are then washed from the marbles and analyzed. (K-4160-5)

# Learning What Pest-Eaters Had for Lunch

When ARS entomologist Matthew H. Greenstone set out to study the diets of some of the natural killers that eliminate crop-destroying pests, he found it no easy job. Predators of the corn earworm, including big-eyed bugs, soldier bugs, spiders, lacewings, and many others, are difficult to study. They're secretive, and they don't feed often.

"In addition, most of them liquify and suck the innards from their prey. You can't simply dissect them to determine what they've been eating," Greenstone says.

Greenstone hopes to increase knowledge of the role that predators and parasites such as spiders and wasps play in reducing insect populations that attack crops. His allies in the war against crop pests are among the newest in technologies.

Ultimately, biocontrol methods may lead to the use of smaller amounts of chemical insecticides. However, to reap this benefit we first need accurate estimates of the impact of natural enemies in reducing pest populations.

Greenstone and biological tech nician Clyde E. Morgan at the ARS Biocontrol of Insects Research Laboratory in Columbia, Missouri, focus on the corn earworm, which is also known as the cotton bollworm and tomato fruitworm. The corn earworm *Helicoverpa zea,* formerly *Heliothis zea,* and the related tobacco budworm, *Heliothis virescens,* are blamed for nearly $2 billion in damages annually to important agricultural crops.

Greenstone borrowed a method commonly used in biomedical research and adapted it to indirectly identify the contents of a predator's stomach. It makes use of hybridoma (monoclonal antibody) technology that can produce large amounts of identical antibodies that are specific to proteins found in the insect pests.

Greenstone and Morgan have published an enzyme-linked immunosorbent assay that uses monoclonal antibodies to detect remains of corn earworm caterpillars in predators' stomachs. The assay is specific and sensitive but has one serious disadvantage: an expensive reader is needed to evaluate the results.

To solve this problem, ARS postdoctoral research associate Melissa K. Stewart developed an immunodot test in collaboration with Greenstone. In performing the test, researchers liquefy individual predators in a mini-blender. The mixture is then used along with the monoclonal antibodies to detect certain proteins of the corn earworm.

"The antibodies we're using are very specific. They have to be specific because we want to distinguish the insect from its close relatives that may live in the same crop field," Greenstone says.

It's quick and accurate: Hundreds of immunodot tests can be done in less than 3 hours. This test requires inexpensive equipment and demands little training to perform and evaluate.

Similar tests are used by medical researchers to identify bacteria and viruses, and to determine pregnancy.

Parasitic wasps also attack the corn earworm. There are several closely related species of parasitic wasps that lay their eggs inside of corn earworm caterpillars. As their larvae develop, they consume the caterpillar.

In this case, researchers could dissect caterpillars and look for eggs and larvae of the parasite under a microscope. "But dissecting insects is tedious and requires special skills," says Greenstone.

C.E. MORGAN

**A spined soldier bug feeds on a corn earworm.**

Stewart and Greenstone have developed a monoclonal antibody that distinguishes larvae of one of the parasitic wasp species from all the others. They are developing a fast immunoassay built around this antibody.—By **Linda Cooke,** ARS.

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# Life-Saving Drugs Could Be Genetically Produced

RICHARD NOWITZ

**Animal physiologist Vernon Pursel at ARS' Reproduction Laboratory prepares an embryo for microscopic examination before implanting it into an animal. (K-4070-17)**

Of the 25,000 hemophiliacs in the United States, nearly 90 percent suffer from forms that are treatable with two costly biological drugs known as blood clotting factors 8 and 9.

What if the nation's entire supply of these blood clotting factors could be produced by one farm animal? And with no more discomfort to the animal than being milked?

While this can't be done yet, the possibility is a lot closer, thanks to ARS scientists at the Beltsville, Maryland, Agricultural Research Center.

Researchers at the center successfully implanted a gene from a mouse into several developing pig embryos. The gene—one that produces whey acidic protein (WAP) in the mouse's milk—has neither beneficial nor harmful effect on the pigs.

But the WAP gene is a useful one for genetic engineering for a couple of reasons. For one thing, it's a gene that pigs don't have; when the protein showed up in the pig's milk, it proved that the mouse gene was in place and had turned on with pregnancy, as it is supposed to do. And the quantity turned out was proportional to the sow's size when compared to the mouse.

Furthermore, by splicing useful genes to the WAP gene, scientists may be able to use it like an on/off switch to produce several biological drugs such as blood clotting factors.

ARS' Robert J. Wall says, "This may be a step toward turning the mammary glands of farm animals

into a source for uncontaminated life-saving medicines." Wall is an animal physiologist with the Reproduction Laboratory at the Center.

Presently, most biologicals approved as blood clotting factors for hemophiliacs and anti-blood clotting factors used to treat heart attack victims are derived from human blood. Supplies of human blood are subject to shortages and concerns about accidental contamination with pathogens.

Wall believes that genetically engineered farm animals could provide an alternative and possibly safer source of these medicines.

The next step may be to splice the key parts of the WAP gene to genes that code for a desired drug, such as factor 9, a blood clotting substance used to treat a form of hemophilia known as Von Willebrand's disease.

Wall says, "Based on our results so far, a single lactating pig could produce enough to treat every known hemophiliac in the United States."

Although the biological drugs are high in the researcher's priorities, other bioengineered products may follow once the technology is developed.

Cow's milk could be altered to contain more of certain proteins. "A 20-percent increase in the right type of casein would be worth $200 million annually to U.S. cheese producers," says Wall.

Another milk protein, lactoferrin, binds iron, which bacteria need to grow. Wall says that genetically increasing lactoferrin in milk might help control mastitis, a bacterial infection of cow's udders that costs U.S. dairy farmers $2 billion annually. More lactoferrin in sow's milk might help reduce anemia in piglets, which accounts for about $9 million a year in medication costs.

Many of the world's adults have difficulty digesting the milk sugar lactose. Reduction of this component in milk would increase the market for milk and milk products. A low-lactose milk would also benefit premium ice cream manufacturers; lactose forms unwanted crystals in their frozen products.

The best animals to produce biological drugs may be cows or goats, Wall says, although he personally favors goats because of their shorter gestation times.

Beltsville lab technician Lea Schulman couldn't agree more about the best animals. "Anybody who has tried to milk a huge lactating pig"—and she has firsthand experience—"would readily attest to the futility of trying to maintain a herd of dairy sows."—By **Vince Mazzola,** ARS.

*Robert J. Wall is at the USDA-ARS Reproduction Laboratory, Bldg. 200, BARC-East, 10300 Baltimore Ave., Beltsville, MD 20705-2350. Phone (301) 344-2362.* ◆

FRED WITTE

**(K-1681-15)**

# Sheep Breeding Gains From Frozen Sperm Technology

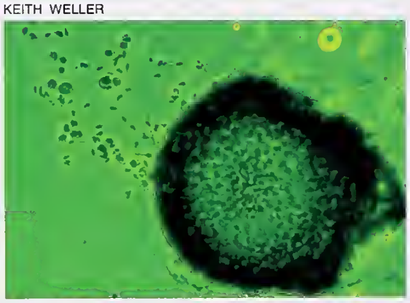
Their long tails quivering furiously, thousands of tiny sperm cells swim across a gray screen. Fernando Rodriguez leans forward slightly, staring intently at the black and white TV in front of him, where the sperm are flagellating.

The screen projects the magnified view of a drop of ram semen, seen under his microscope.

"I give this one a 3.5," says Rodriguez, carefully noting the number in his notebook. "That's out of a possible 4—the top score."

Unlike an Olympic judge, he's not interested in form or style of the tiny swimmers. Instead, he's looking at how many of the sperm can still move their tails after being thawed from a deep freeze. The score—commonly referred to as percent motility—estimates the percentage that are still alive.

KEITH WELLER



(K-4165-7)

Rodriguez, an animal scientist at the ARS Range Sheep Production Efficiency Research Unit in Dubois, Idaho, studies how gel from the common aloe vera plant can help sheep sperm survive a stint in liquid nitrogen. At –196°C, liquid nitrogen holds sperm cells in suspended animation until they're needed at a later date.

It's all part of a new scheme to help make artificial insemination (AI) feasible for sheep.

Freezing and storing of semen and AI are already widely used by dairy and some beef cattle producers. The practices enable them to save sperm from superior animals with desirable genes for a timed insemination with good breeding animals. Ranchers can thus replicate traits from superior animals in other herds and selectively breed their own flocks to boost desirable characteristics.

The procedure also helps prevent diseases that are spread through sexual contact.

For sheep ranchers, the technique could cut the number of rams needed for breeding on the farm.

"Ultimately, we hope to boost lamb production, which could make lamb more plentiful and less expensive for consumers," says Rodriguez.

However, AI isn't as easy in sheep as it is in cattle. Ram spermatozoa don't survive freezing as readily as those from cattle, possibly because the cell membrane isn't as rigid. And a ewe's cervix (the narrow outer end of the uterus) has folds that form blind, dead-end loops, an arduous trip for even the most hardy sperm.

Research at the Dubois lab addresses both problems—by taking special care to protect the sperm during freezing and by placing the semen directly in the ewe's uterus with a simple surgical procedure.

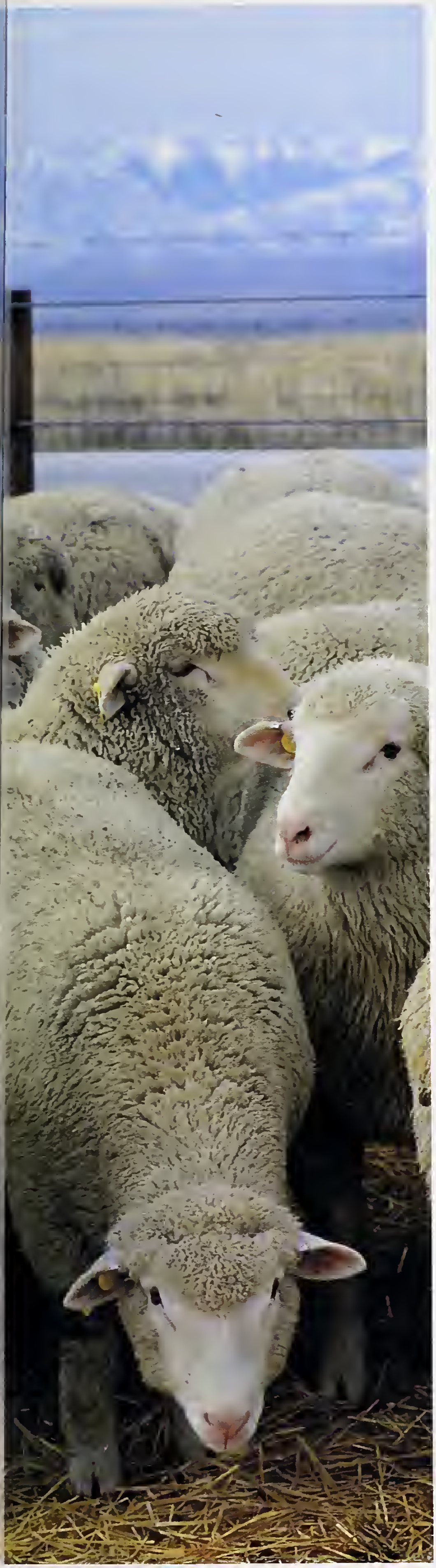
Rodriguez experiments with semen diluents—substances added to the semen before the deep-freezing process—and other tactics to enhance the sperm's survivability.

He's found that the pulpy gel from the aloe vera plant—long touted for its healing properties—makes an ideal diluent component in extenders for semen. Because ram semen is so concentrated (between 1 and 3 billion sheep semen per milliliter, compared to 800 to 1200 million in bulls), the researchers dilute it before freezing, so it can be used for several insemination doses.

Rodriguez first hit on the idea of using aloe vera while he was a graduate

KEITH WELLER

**Research flock at the U.S. Sheep Experiment Station near Dubois, Idaho. (K-4166-5)**



student at Sul Ross State University in Alpine, Texas. One of his professors had found that aloe vera offered promise for treating certain vaginal and uterine infections in cows. But a shortage of infected cows prevented further studies to test the theory.

Meanwhile, Rodriguez noticed that cattle sperm mixed well with the gel, so he began testing it as a diluent for freezing semen.

The goal, says Rodriguez, was to find an extender to use in developing countries.

"Aloe vera grows naturally in 75 percent of lesser developed countries," he notes.

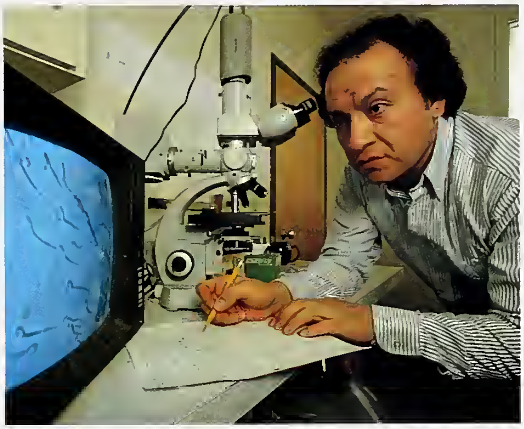
He later entered a doctoral program at New Mexico State University and switched from cattle to sheep. A nursery in Texas donated several aloe plants, which Rodriguez cultivated on campus for 2 years during the study.

After harvesting, he split the thick, fleshy leaves down the middle—"it's sort of like skinning a fish"—and collected the clear, gooey gel from the plants.

"Aloe vera gel contains glucose, fructose, and other plant sugars that may serve as an energy source for the sperm," says Rodriguez. "Because the gel is so viscous, it seems to coat and protect the cells during freezing."

Researchers elsewhere have experimented with other unusual extenders, such as coconut milk or tomato juice, which also contain plant sugars. While they preserve semen at room temperature for a time, none work as well as aloe vera gel for freeze protection, says Rodriguez.

KEITH WELLER



**Animal scientist Fernando Rodriguez checks survival of ram spermatozoa that have been frozen in liquid nitrogen, then thawed. (K-4163-6)**

Other extender additives included with the aloe are egg yolk, which protects sperm during the cool-down period before freezing, and glycerol. After months of refining the ratios of the extenders, Rodriguez found that a diluent made of 40 percent aloe vera "works very well." The rest of the diluent, about 50 percent, is sodium citrate, a common buffer solution.

Rodriguez also adapted a pellet-freezing technique for the semen that's used for cattle semen in some parts of the world.

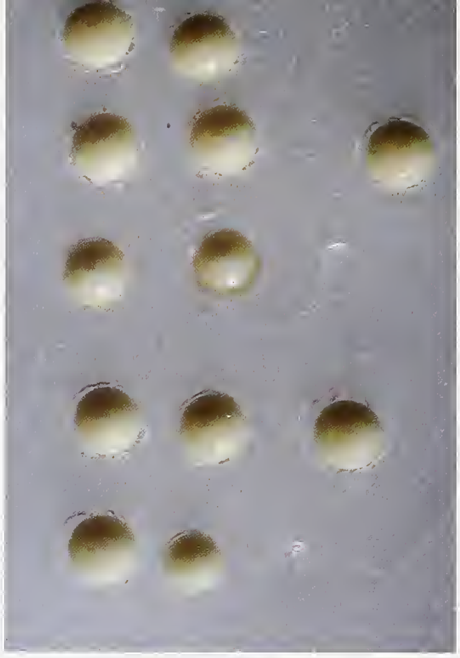
After mixing in the diluents, he hoists a large block of dry ice onto the lab bench. He presses the end of a small, blunt metal rod into the block to form button-sized indentations in the ice. Next he places 3 to 5 drops of the semen mixture into the holes, where it quickly freezes, forming small, round pellets. These are scooped up and placed in plastic goblets, which are then lowered into a tank of liquid nitrogen.

Rodriguez tests the sperm's progressive motility—gauged by the score of 0 to 4—before freezing and after thawing.

"An excellent sample usually has between 50 and 65 percent of healthy sperm cells after thawing," says Rodriguez.

Many semen samples, however, have considerably fewer survivors—between 20 and 50 percent, depending on breeds, rams, and time of year when the semen was collected.

One possible explanation for some semen's poor freezability relates to levels of naturally occurring chemicals,



KEITH WELLER

Pellets of ram semen frozen on dry ice. (K-4165-10)

called polyamines (PA's). Several studies from other labs suggest that one PA, spermine, is important for keeping other cell membranes stable. Low levels of PA's would likely contribute to a poor, post-thaw survival, Rodriguez reasoned.

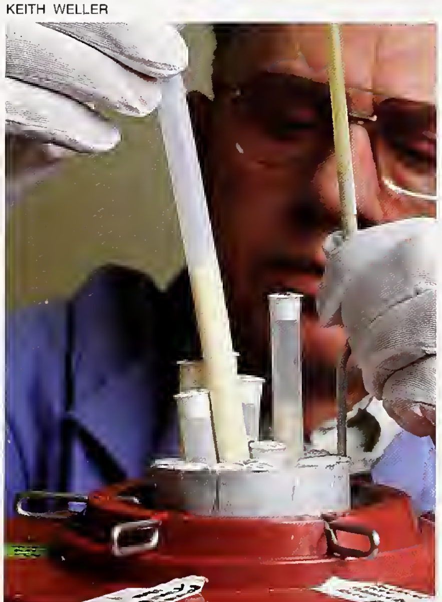
Rodriguez hopes to learn whether boosting polyamine levels will enhance freezability. Initial results show that washing off the seminal plasma from the sample and adding spermine to the diluent appears to help frozen-thawed sperm swim farther and faster.

Researchers determine those values with the bovine cervical mucus test, which measures the distance sperm can travel during a set time (90 minutes, in this case) through a tube of the mucus. The procedure is also used in clinical medicine to study human fertility.

Helpful as it is, the cervical mucus test is no more than a model. The ultimate objective is to identify sperm that successfully impregnate ewes.

To that end, the Dubois scientists have adopted a technique known as laparoscopic AI that allows AI technicians to see inside a body. Laparoscopes are narrow, flexible or rigid tubes equipped with optics and a light source that allow veterinary surgeons to see inside a body. In surgery on humans, similar instruments are used to simplify invasive procedures.

KEITH WELLER



Technician Bill Gardner stores semen pellets in liquid nitrogen. (K-4162-1)

"By inseminating ewes this way, we not only bypass the cervix, we also avoid having to take the ram's changeable sex drive into account," says John N. Stellflug, an animal physiologist who performs the techniques at the Dubois station. During the summer months, for example, the ram's libido tends to wane and natural breeding becomes less efficient.

The scientists treat the ewes with hormones to stimulate ovulation—the release of an egg from the ovaries. Just before the AI procedure, Stellflug gives the sheep a mild tranquilizer.

He places the ewe on her back in a cradle to expose her abdomen. After applying a local anesthesia, Stellflug makes a small incision—about the width of a little finger—to insert the laparoscope.

"If we just want to check whether a certain ewe is ready to be bred, we use the laparoscope to visualize the uterus, the ovaries, and the corpus luteum—which forms when the ovarian follicle ruptures to release an egg," says Stellflug. "That tells us we have a reproductively active ewe."

But he doesn't actually look at the ovaries during the AI procedure. That's to avoid interfering with ovulation, which could occur after insemination.

Stellflug pokes a tiny needle in the uterus and delivers a few drops of semen—containing about 25 million sperm—to each of the two uterine horns.

The entire procedure lasts under 3 minutes. Afterwards, Stellflug sprays the tiny cut with an antibiotic. No stitches are needed. He gently shifts the ewe from the cradle to her feet, and she trundles off to rejoin the flock.

"We've inseminated over 3,000 ewes with frozen, then thawed semen in the past 2 years," says Stellflug. "The proportion of successful pregnancies from these artificially inseminated sheep is close to 70 percent. That's similar to that of natural mating during the mature ewes' first heat—or estrus cycle—in the breeding season."

Although the cost of laparoscopic AI makes it impractical for the average sheep producer to use at this time, researchers find it a valuable tool.

"Perfecting AI in sheep could help U.S. sheep producers in several ways," says Rodriguez. "We could more easily use frozen semen from breeds around the world to incorporate desirable genes into our flocks here. Also, by saving semen from their best rams, ranchers could improve their own flocks."

A few producers in the United States are using AI to introduce genes from foreign breeds like the Booroola Merino from Australia. Booroola ewes carry a dominant gene for high ovulation rates. Introducing their genes into flocks is a possible way to boost production, says Stellflug. Other Merino varieties from Australia are known for their fine, soft wool.—By **Julie Corliss,** ARS.

*Fernando Rodriguez and John N. Stellflug are at the USDA-ARS Range Sheep Production Efficiency Research Unit, U.S. Sheep Experiment Station, Dubois, ID, 83423. Phone (208) 374-5306.* ◆

# High Hopes for Seeding Guayule

"We get 10 times more seedling survival with our method."

JACK DYKINGA

(K-1559-3)

Home-grown guayule, a native shrub that produces natural rubber, would reduce our dependence on imports of this critical material while boosting farm income in the southwestern United States. This 3-foot tall, drought-tolerant shrub, pronounced why-YOU-lee, grows wild in southwestern Texas and northern Mexico.

The surest way to get a field of guayule established is to transplant seedlings that were started in greenhouses. But while this expensive, labor-intensive planting technique is practical for high-value crops like tobacco, it's not economical for domestic rubber production.

Agricultural Research Service scientists in Arizona developed a seeding technique that produces stands that are more uniform, with seedling growth more vigorous, than ever before. The technique promises to make guayule more competitive as a new crop for U.S. growers.

"Compared to scattering seed on the soil surface, we can get 10 times more seedling survival with our method, and it's now practical on a large scale," says Francis S. Nakayama, a chemist at the U.S. Water Conservation Laboratory, Phoenix, Arizona.

First, the seeds are pre-treated with a combination of polyglycol, growth regulator, and fungicide. Then a commercially available planter seeds them to a depth of one-quarter to one-half inch. The seed treatment procedure was developed by Ganapathy R. Chandra at the Plant Hormone Laboratory in Beltsville, Maryland.

"Seeds planted one-quarter-inch deep can be covered with soil, while a thin covering of vermiculite works better for the deeper planted seeds. This is presumably because vermiculite presents less resistance to emerging seedlings than the soil covering," says Nakayama.

ARS scientists worked with collaborators from state agricultural experiment stations of the University of California, Texas A&M University, New Mexico State University, and the University of Arizona to breed improved guayule plants. They have succeeded in doubling natural rubber production from about 500 pounds per acre per year for native plants to 1,000 pounds for the new breeds.

These advances will help make guayule a commercial crop that can compete with natural rubber harvested from trees in southeastern Asia. It would also be an alternative crop for farmers in southwestern states under both irrigated and dryland farming operations.

Producing natural rubber in the United States would lessen our reliance on imported rubber and ensure steady supplies of this vital industrial raw material. Climatic or political changes overseas might affect imported rubber's availability and price.

Some economic studies indicate that there will be a natural rubber shortage worldwide by the mid-1990's and certainly by the turn of the century. Today's popular radial tires require more natural rubber than older bias-belted types. Aircraft tires, which operate under high temperatures and impacts, require almost 100 percent natural rubber.

Natural rubber is a renewable resource while synthetic rubber is manufactured from petroleum—a resource that is steadily being used up.—By **Dennis Senft,** ARS.

*Francis S. Nakayama is at the USDA-ARS U.S. Water Conservation Laboratory, 4331 E. Broadway, Phoenix, AZ 85040. Phone (602) 379-4356.* ◆

JACK DYKINGA

JACK DYKINGA

**Harvesting guayule. (K-1557-7)**
**Below: Rubber extracted from dried guayule plants. (K-1633-1)**

# Apples Without Bruises

## Bashless bagging makes for a prettier fruitbowl

The key to fewer bruises on apples coming out of a packinghouse may be in the bag—or at least in the method used to bag the fruit.

Agricultural Research Service scientists have designed a bagging machine that cuts bruises in Golden Delicious apples to about a fifteenth of that caused by other bagging machinery.

Studies of the 1986-87 average Michigan apple crop indicated that 91 to 95 percent of a 3-pound bag of Golden Delicious apples was battered by the bagger. There were 2.75 bruises per apple.

Dale Marshall, an agricultural engineer in the Fruit and Vegetable Harvesting Unit at East Lansing, Michigan, was convinced that the machinery used to bag the fruit was responsible.

Conventional baggers gather apples in a pan that is inclined at about a 10-degree angle. The filled pan is tipped to nearly a 60-degree angle and the apples are dumped into a polyethylene bag. Marshall believed that this action was causing many rear-end collisions as the apples fell into the bag.

With a 35-mm camera in hand, he set out to prove his theory. Photographs confirmed that instead of falling into the bag en masse, as proponents of the conventional packing method claimed, the apples were bumping into each other, causing the fruit to bruise.

"I took pictures that showed apples separated by 2 to 4 inches, and when one stopped, the others didn't," he says.

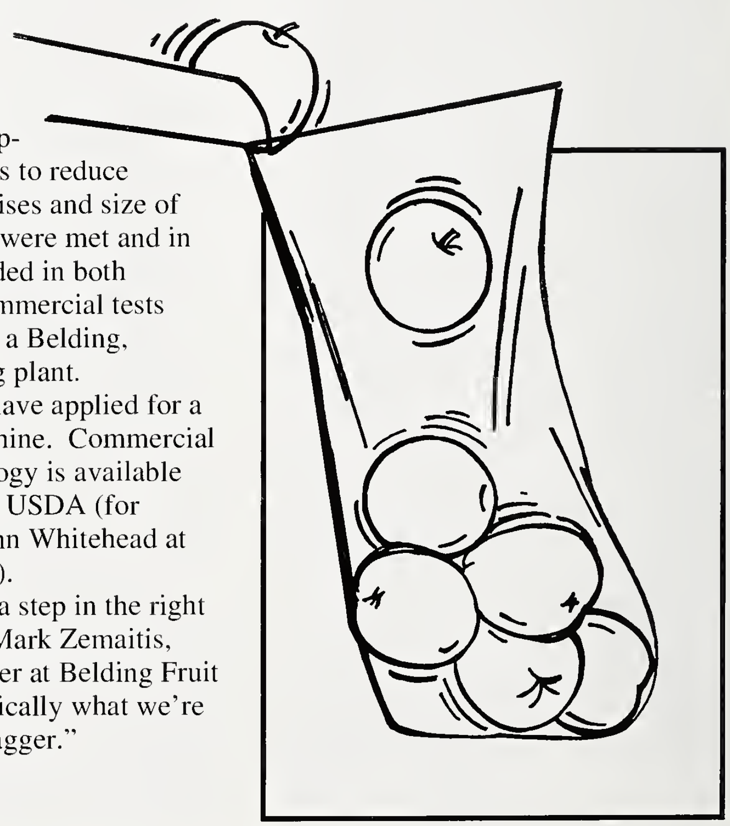
"Our research has found that the bagging operation was causing more bruises than any other single apple-handling operation between the orchard and local grocery store," Marshall says.

Armed with this knowledge, Marshall and co-workers set out to design an automatic bagger that would maintain the standards needed for apples to be labeled U.S. Extra Fancy grade—less than one bruise per apple and the battered area can be no more than 0.2 of a square inch (about half the size of a quarter).

In an effort to reduce the number of these collisions, engineers have simply enclosed the pan with a bag. The apples roll into the pan, the pan/bag combination is then tipped until the bag of apples is almost vertical, and it is encased in a cushioned box.

The box holds the apples in place as the pan is removed, allowing the apples to settle, but not fall. Finally, the box moves the bag to a closer and the bag slides away.

This new machine, the "low-damage automatic apple bagger," proved successful, essentially eliminating the high-velocity blows to the fruit that are experienced with conventional equipment. So the goals to reduce the number of bruises and size of the battered areas were met and in some cases exceeded in both laboratory and commercial tests earlier this year at a Belding, Michigan, packing plant.



The inventors have applied for a patent on the machine. Commercial use of the technology is available for licensing from USDA (for details, contact Ann Whitehead at the address below).

"It's definitely a step in the right direction," notes Mark Zemaitis, production manager at Belding Fruit Storage. "It's basically what we're looking for in a bagger."

Zemaitis says he's encouraged by the invention; however, a smaller, quicker machine would be more desirable.

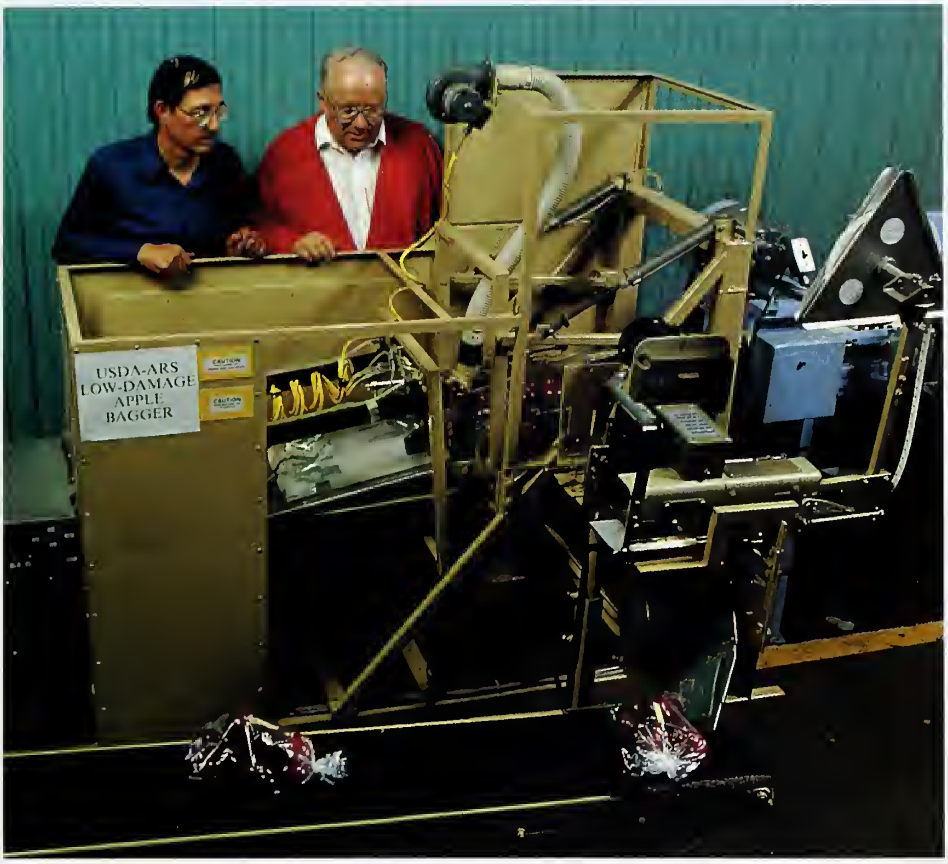
While the new technology is slightly slower than traditional bagging machines—filling about 7 bags each minute compared to the industry average of about 10—Marshall is optimistic that improvements will bring the bagger up to speed.

"It's 30 percent slower right now, but you can't have your cake and eat it too without additional research," says the agricultural engineer.

The reduced speed may be a reason that some packing plants opt to modify existing machinery rather than purchase new equipment.

Before developing the new bagger, Marshall modified an existing commercial automatic bagger using design improvements that could easily be adapted to other baggers. The work was conducted through a Cooperative Research and Develop-

KURT STEPNITZ




**Apples move through an ARS developed, low-bruise apple bagger as agricultural engineer Dale Marshall (right) and technician Dick Wolthuis put the machine through its paces. (K-4190-1)**

ment Agreement with Powell Manufacturing, in Faber, Virginia.

The basic idea is to reduce the kinetic energy in the apples during bagging. The first change was to reduce the angles of the accumulation pan and the bag holder.

Then, using various materials, Marshall cushioned the end and bottom surface of the accumulation pan to protect the apples as they rolled into the pan. Additional cushioning was placed under the wicket of bags to soften the blow to apples when the wicket is nearly empty.

During filling of about 100,000 bags with the modified machine, researchers noted an 80-percent reduction in the number of bruises per fruit and the total size of the bruised area. Production speed was not affected by the modifications.

Marshall says that reducing bruises on apples will benefit both the industry and the consumer.—By **Marcie Gerrietts,** ARS.

*Dale Marshall is with the USDA-ARS Fruit and Vegetable Harvesting Research Unit, Room 208, Farrall Hall, Michigan State University, East Lansing, MI 48824. Phone (517) 353-5201. For patent licensing information, contact Ann Whitehead, Room 403, Bldg. 005, BARC-West, 10300 Baltimore Ave., Beltsville, MD 20705-2350. Phone (301) 344-2786.* ◆

BOB BJORK

**(K-2908-1)**

## "Apple" Computer Records Impacts

An instrumented sphere, developed by ARS and Michigan State University scientists in 1986, can give apple packinghouses answers to their bruising problems in as little as 1 hour, says Galen Brown, head of ARS' Fruit and Vegetable Harvesting Research Unit at East Lansing, Michigan.

Researchers built an electronic sphere that records the duration, time, and magnitude of impacts to the fruit during the packing process. A database has been developed from information recorded by this apple computer and information from bruises caused on real apples. This database can assist packinghouses with selection of machinery, cushioning materials, packing materials, and transportation to maintain quality fruit.

The computerized sphere was used on conventional apple baggers to determine the magnitude of bruising and where in the process the fruit was being bruised. As the new bagger took shape, the sphere was used to make certain that bruising was actually reduced.

The commercially available sphere is currently being used throughout the world to reduce bruising on an array of fruits and vegetables, including tomatoes, peppers, sugar beets, and potatoes.—**Marcie Gerrietts,** ARS.

# Natural Enemies Gang Up on Corn Pests

KEITH WELLER

**Entomologist Leslie Lewis checks for dead European corn borers in corn stalks that contain a biological control fungus. (K-4130-12)**

A half century ago in the Midwest, cornfields in terrible condition were a common sight. Strewn and littered with broken stalks, these unproductive fields had fallen victim to the combined ravages of an insect, the European corn borer, and microorganisms that cause stalk rot.

While farmers applied chemical insecticides to cope with the problem, microbiologists searched for antibiotic remedies and plant geneticists redoubled efforts to breed resistant corn.

Despite progress, European corn borers still cause an estimated $400 million loss each year. The nation's farmers spend another $50 million to control the pest, says Leslie C. Lewis, an ARS entomologist at Ankeny, Iowa. Estimates on losses from stalk rot generally range even higher.

To reduce corn losses and the prospect of pests developing resistance to chemical controls, scientists hope to marshal several natural forces. Among these are borer-killing insects and microbes such as *Beauveria bassiana* and *Nosema pyrausta* that kill borers. Another beneficial microbe, *Gliocladium roseum,* feeds on stalk rot fungi.

"We're exploring the interactions in nature to find ways of improving control," says Lewis. "We can't depend on one method by itself to control a pest indefinitely."

Traditionally, the front line of defense against lodging (stalks flattened to the ground) has been breeding corn with stalk strength and resistance to stalk rots and corn borers. Scientists are always looking for these traits among newly acquired strains of corn and related plants, says agronomist Mark J. Millard who is with Iowa State University at Ames. Millard is curator for more than 9,000 strains of corn at the ARS North Central Regional Plant Introduction Station on the university campus. About a thousand new accessions are added to the collection each year.

Leaves of some modern corn hybrids contain enough of a compound called DIMBOA to keep each year's first generation of corn borers from doing much damage, says Lewis. It's the second generation hatching in midsummer that poses the greatest threat as larvae eat tunnels in the stalks. The concentration of DIMBOA in corn that has nearly reached its mature size is not great enough to do much good.

Another line of defense besides breeding is gaining popular appeal—applying a bacterial insecticide called Bt (*Bacillus thuringiensis*). ARS scientists were first to show that Bt does a good job killing corn borers when it is applied to corn just as larvae of the second generation begin to hatch. Bt produces crystals that are toxic to corn borers but harmless

to helpful insects, earthworms, birds, and mammals.

A major seed corn company, trying to ensure the safety of its detasseling crews, depends heavily on Bt, avoiding exposure of detasseling crews to chemical pesticides. Detasselers are workers who are transported through fields to strip the male organs from corn plants, preventing self-fertilization of the female portion of the plants. Farm workers apply granules of Bt spores and crystals over the whorl—curled top center leaves—of the plant. Usually, toxicity to the corn borer begins to break down within 24 hours.

Experimentally, at least one biotech company has transformed another bacterium to produce a longer-lasting Bt toxin. And another firm has transformed a bacterium to produce the toxin while living in the seed and the borer's home—the plant's vascular system.

Although genetic diversity from among thousands of *Bt* strains might be tapped for this corn borer control strategy, Lewis says, "We've yet to learn how quickly borers may develop a tolerance to the toxin if Bt is in every corn plant."

European corn borers cause an estimated $400 million loss each year.

Through research to improve alternative control strategies, Lewis says, corn growers won't be tempted to "put all their eggs in one basket" and Bt resistance might develop more slowly. His research is now focused mainly on the fungus *Beauveria bassiana* that is entomopathogenic—it

KEITH WELLER

**Corrugated paper rings, such as this one held by entomologist Leslie Lewis, form pupation sites for corn borer larvae during laboratory rearing. (K-4131-5)**

makes corn borers sick. Like the bacteria that were genetically engineered to produce Bt toxin, *B. bassiana* is an endophyte; it lives inside the corn plant.

"*B. bassiana* has probably been around since dirt," Lewis jokes. The endophyte occurs naturally and has a history of partial effectiveness in keeping corn borers in check. Once scientists find ways to improve how *B. bassiana* works in an ecosystem with other natural controls, it's expected the pest will develop only a little resistance, if any.

As with other entomopathogens, *B. bassiana* often suppresses pest populations, most noticeably after a crop may have already become severely damaged. But in a recent 2-year study, Lewis, along with co-worker Lori Anderson Bing, put corn grits sprayed with a suspension of *B. bassiana* conidia—spores—into the corn whorls before tasseling. By early August, the fungi penetrated the plant's vascular system and reduced season-long corn borer tunneling in stalks about one-third.

Now in the ARS teacher fellowship program, Bing and graduate student Bruce L. Wagner hope to gain insights on how the fungus moves into corn's vascular system. The study may help scientists find ways to enhance corn borer control.

The researchers would like to learn what roles plant genetics, allelochemicals, and other naturally occurring microbes may have in fostering or hindering the populations of *B. bassiana* and corn borers.

Some light has already been shed on interactions of corn genetics, stalk rots, and corn borers in a 2-year study on six corn hybrids by plant pathologist Charles C. Block of Iowa State and three ARS scientists recently retired or reassigned from Ames and Ankeny.

Two weeks after the hybrids were in full bloom, the scientists inoculated corn-borer-infested stalks and healthy stalks with a mixture of spores from fungi that cause Iowa's three most prevalent stalk rots. Then, in early fall, they found that corn borers increased severity of the stalk rots. But which species of pathogenic microbes had become most prevalent depended on which corn hybrid was involved.

Although the study was focused on yield-robbing pathogenic fungi, Block notes that breeding corn to resist borers could help prevent wounds to the plant. Wounds provide passageways for nonpathogenic microbes to enter the plant and weaken stalks as they digest cellulose.

Corn genetics may also have a great impact on how well several fungi that are mycoparasitic—eat other fungi—thrive as endophytes in plant tissue, says ARS plant pathologist Nader G. Vakili. In a field experiment at Ames, he found that one corn line produced a 65 percent greater yield when protected by the mycoparasite *Melanospora*

KEITH WELLER

European corn borer larvae. (K-4126-11)

*damnosa.* In a 4-year study he found that populations of *M. damnosa* steadily built up in a field of continuous corn.

In Central America, disease development is suppressed in indigenous corn intercropped with pole beans, says Vakili. Within inbred corn lines in the ancestry of Corn Belt hybrids, there seems to be a mechanism for control that can be expressed in varying degrees. And as the desired corn genes are identified, they may become valuable to breeders. State agricultural experiment stations have developed 2 inbreds that most often seem to favor beneficial microbes: B73 and M017.

KEITH WELLER

Iowa State University entomologist David Orr examines a corn stalk. (K-4125-5)

In an experiment intended for studying mycoparasites, Vakili planted B73 in a field highly infested with the entomopathogen *B. bassiana.* In the fall, he found one-fourth of the stalks supporting *B. bassiana.* Vakili says, "To me that suggested the seeds I was using had some heterozygous recessive gene that made the stalks amenable to the fungus."

Vakili found that *B. bassiana* does more than kill corn borers as he looked through his microscope at a newly hatched saprophytic nematode—a nematode that scavenges bacteria and spores of fungi inside plants. Crawling across an agar plate, the nematode backed off from a colony of *B. bassiana* "like it burned its snout."

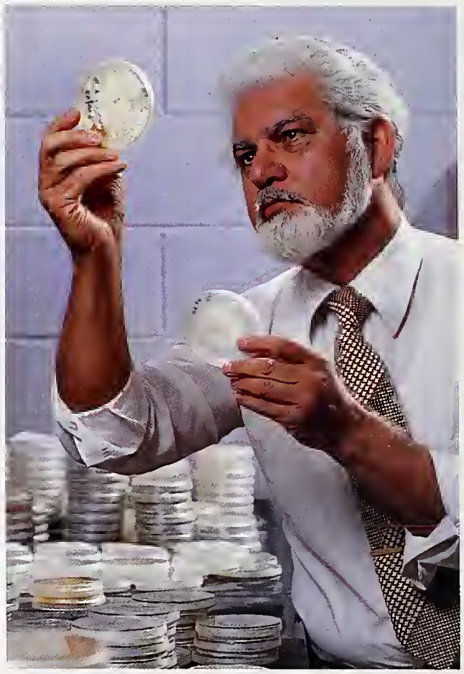
With a glass needle, Vakili placed some nematodes in the middle of *B. bassiana* colonies. A day or two later the nematodes "just stuck straight up like telephone poles," Vakili says. "They were dead."

In research at the National Soil Tilth Laboratory, Ames, Vakili is now trying to learn what effect *B. bassiana* in the soil may have on earthworms.

Studies by Vakili and Lewis have shown that the vascular systems of some corn hybrids abound with *B. bassiana* as long as the plants remain green.

Two more nemesis of corn borer, the funguslike microsporidium *Nosema pyrausta* and a tiny wasp called *Macrocentrus grandii* are being researched by Lewis and Iowa State entomologist David Orr. The wasp deposits a single egg into a borer larva before it bores into a corn plant. The single egg is destined to become 25 wasp larvae. If the borer larva happens to be diseased with *N. pyrausta,* the new wasp larvae also become diseased.

KEITH WELLER



**Plant pathologist Nader Vakili checks fungal samples. When used in combination, *Beauveria bassiana* and *Sphaeronaemella helvelli,* sharply reduce damage caused by second-generation European corn borers. (K-4133-8)**

There is concern, however, that the two beneficial insects may work against one another. Diseased corn borer larvae parasitized by *M. grandii* fail to spin cocoons that are needed by the wasp larvae to provide scaffolding for their own cocoons. Without the security of a cocoon, the wasp larvae may become nervous, wander from their source of food, and die.

Sick corn borers suffer from a wanderlust of their own, as Orr learned by training a videocamera on the insects. Larvae that were infected or parasitized became irritable, moving 10 times as much as healthier borers. Larvae that were both infected and parasitized moved 50 times as much.

Orr and Lewis also found that the wasp could transmit disease from infected to healthy borers. They hope to test whether they can release laboratory-raised wasps that will be able to transmit the disease and thereby double the impact of the wasps or the disease alone.—By **Ben Hardin,** ARS.

*To contact scientists mentioned in this article, write or telephone Ben Hardin, 1815 North University St., Peoria, IL 61604. Phone (309) 685-4011.* ◆

# Thwarting One of Cotton's Nemeses

There's not much good to be said for the pink bollworm, cotton's most destructive pest, except that it is being controlled to cut crop damage.

Scientists have developed strategies, such as increasing native populations of predatory insects and pest-resistant cotton varieties.

Thanks to research, growers today can also use cultural practices such as early plowdown of harvested cotton to break up stalks and bury overwintering pink bollworms. And they can disrupt normal mating by releasing sterile insects and using copies of natural compounds, called pheromones, that the pink bollworm uses to attract mates.

Such strategies, together with judicious use of insecticides, put together in various combinations, form what is called an integrated pest management system.

"Less than 20 years ago, cotton growers routinely sprayed 12 to 15 times with insecticides during each growing season. Today, they sometimes get by with five or six applications, in large part because of research at the Agricultural Research Service's Western Cotton Research Laboratory in Phoenix, Arizona, and at cooperating universities," says Thomas J. Henneberry, entomologist and laboratory director.

The pink bollworm has destroyed annually an estimated 25 percent of the cotton crop in California's Imperial Valley. In infested areas, that can represent a loss exceeding $190 per acre.

The damage is from larvae that feed on flowerbuds and bolls of cotton plants. There can be up to five generations of the pink bollworm in one 6- to 8-month cotton-growing season. The insect's world distribution, voracious appetite, and enormous populations cause tremendous damage to cotton crops.

**Trap for pink bollworm moths. (K-4113-1)**

The pink bollworm, *Pectinophora gossypiella (Saunders)*, was first identified in 1843. It has been a destructive pest of cotton in most cotton-growing regions of the world since the early 1900's. But it wasn't until the 1950's that scientists, in an effort to control outbreaks, started looking at its biology.

"When I started genetic studies in 1967, I was looking for ways to manipulate the insect's reproduction or its behavior, hoping to make it a candidate for an autocidal or self-killing control method," says Alan C. Bartlett, ARS geneticist at the Phoenix laboratory.

Such methods include releases of millions of males that are genetically sterile. These sterile males mate with native females in cottonfields, and these mated females produce sterile eggs. This is how the pink bollworm is now controlled in the San Joaquin Valley of California.

"We discovered that there was a high degree of genetic variability in the pink bollworm, which is good because the more variability, the greater chance we'll find some genetic flaw we can use against the insect. On the downside, the greater variability makes it more difficult to manipulate the insect's genetics. It also means the insect can adapt more quickly to environmental changes and insecticides, thus surviving," Bartlett says.

As is often the case in plant breeding, plants that have natural insect resistance lack other desirable properties such as high yield. Cotton breeders get around this by crossing bollworm-resistant plants with several generations of plants that

TIM MCCABE

**Cotton boll sliced open to expose damage from a pink bollworm. (K-2886-13)**

possess other desirable properties, says F. Douglas Wilson, plant geneticist at the laboratory.

In a 3-year study at two locations, one such advanced-generation hybrid, when compared to a control variety, required 41 percent less insecticide to control pink bollworm and yielded 12 percent more lint. It was also significantly earlier maturing, which reduces risk of damage caused by early fall rain and reduces the number of overwintering worms to infest next year's crop.

Parasitic and predatory insects of the pink bollworm also offer control. The newest parasite—*Trichogrammatoidea bactrae Nagaraja*—kills bollworm eggs before they can hatch into larvae. *T. bactrae* will probably adapt to the climate of the southwestern desert, which it is similar to its native Australian habitat.

"Our laboratory studies show the parasite is active at temperatures ranging from 60°F to 90°F. Females emerge and lay their eggs inside bollworm eggs within 24 hours. The *T. Bactrae* insects are nourished by eating the contents of the eggs," says William D. Hutchison, an entomologist formerly with the laboratory.

### Unnatural Mating Attraction

ARS entomologist Hollis M. Flint and technician John Merkle were able to alter the response of male pink bollworms so they seek unnatural ratios of two components found in their pheromone. This is the first time an unnatural ratio of pheromone compounds has been made that males prefer over the natural ratio emitted by females.

Flint and Merkle accomplished this by releasing only one of the component isomers into cottonfields. This alters the signal received by the male's antennae. Females that produce the natural ratio of component isomers are no longer able to chemically communicate with males. Instead, males are attracted to unnatural combinations of the two isomers, which may be presented in traps or killing formulations.

ARS and university scientists determined in 1973 that the pheromone of pink bollworm is about a one-to-one mixture of the Z,Z and Z,E-isomers of 7,11-hexadecadien-1-ol acetate.

Known as gossyplure, it has been used to disrupt normal mating by placing numerous dispensers of the pheromone in the field to compete with females. Scientists theorize that males go on wild goose chases following the artificial pheromone dispensers and never locate a female. Also, males may become overwhelmed by massive doses of the pheromone released into the air, perhaps blocking their antennal receptor sites and central nervous systems.

The new attractive formulation contains a 9-to-1 ratio of the Z,Z- to Z,E-isomers. Males are especially attracted to this ratio of isomers in a field treated with the Z,Z-isomer, ignoring females along the way. The new system can be used to treat cottonfields and monitor populations with traps baited with the unnatural ratio. The scientists have patented the control and monitoring system and have shown the same methods work for pinkspotted bollworm, a related moth that is a problem in Australia.—By **Dennis Senft,** ARS.

*Scientists mentioned in this article are at the USDA-ARS Western Cotton Research Laboratory, 4135 East Broadway, Phoenix, AZ 85040. Phone (602) 379-3524.* ◆

# Trapping Bugs With a PVC Pipe

In the curious world of bug traps, the maxim is always the same: Capture insects and prevent their escape.

But while that seems fairly obvious, success is equally measured by design and function. A cardboard trap that breaks apart in the rain, for instance, will not accomplish the mission. The same is true for traps that are too small, too awkwardly built, or don't dispense their attractants properly.

At ARS' National Center for Agricultural Utilization Research, in Peoria, Illinois, scientists may have come up with a trap that avoids those limiting factors while maximizing the potential for luring bugs.

Entomologists Patrick F. Dowd and Robert J. Bartelt, along with microbiologist Donald T. Wicklow, have designed a field trap that is sturdy, simple and, so far, quite successful. Made of tough PVC pipe, the white T-shaped trap measures 9x14 inches fully deployed and stands up to harsh weather. One end of the horizontal pipe is screened at two points to protect the bait; the other contains a funnel through which the insects, typically beetles and mosquitoes, enter.

Once inside, the insects do not turn around and exit through the funnel opening, says Dowd, "because they're stupid. Rather than go out the same way they went in, they look around for daylight and follow that to try to escape." In this case, they follow the light down the vertical pipe and end up caught in a clear plastic bag.

Once inside, the insects do not turn around and exit through the funnel opening—because they're stupid.

SCOTT BAUER

**Tough, weather-resistant PVC traps for flying insects such as beetles and mosquitoes. (K-3805-4)**

Because many commercial traps are fixed in one position on trees or posts, the odors of the bait attractants that lure pests are not evenly released over a large area. The scientists adjusted for that problem by attaching a swivel mechanism to wire at the top of the T-trap and a small metal plate to one side of the horizontal pipe. The two attachments help to orient the trap into the wind, allowing for an even and continuous rate of release of the bait odor.

"We wanted to include both of those things because the way an insect flies to an attractant is always upwind," Dowd says. "By orienting in the wind, this trap helps the insect detect the attractant, fly up to it, and easily enter the funnel."

The trap was designed primarily to capture dusky sap beetles and picnic beetles. Both are pests of sweet corn, fruits and other vegetables and both can transmit *Aspergillus flavus,* the fungus that produces aflatoxin. But the trap has also snared moths, bees, yellowjackets, and mosquitoes.

Dowd says the trap would probably work well in home gardens if gardeners were to bait it with the appropriate attractant for a particular insect.

The researchers are patenting the trap and expect to develop a standard odor for sap beetles that will release evenly for about a month without replenishment.—By **Matt Bosisio,** formerly ARS.

*Patrick F. Dowd, Robert J. Bartelt, and Donald T. Wicklow are at the USDA-ARS National Center for Agricultural Utilization Research, 1815 North University, Peoria, IL 61601. Phone (309) 685-4011.* ◆

# Irrigation Tubing Does Double Duty

Farmers who grow crops under furrow irrigation could save hours of labor every time they water, using a new modification to layflat irrigation tubing recently patented by ARS agricultural engineer Allan S. Humphreys and Ed E. Oest, an irrigation equipment engineer and dealer from Fruita, Colorado.

Use of the modified tubing holds promise for conserving water, reducing leaching of agricultural chemicals, and in some cases, reducing erosion, says Humphreys, who is at the ARS Soil and Water Management Research Unit in Kimberly, Idaho.

Such problems concern farmers who raise crops on the 58 million acres of irrigated land throughout the United States. About 40 percent of that land is watered by furrows—the shallow trenches dug between crop rows that channel water across fields.

Regular layflat tubing, made with flexible materials such as rubber, vinyl, or polyethylene- or vinyl-reinforced polyester has been used by farmers for years. It's a low-cost alternative to rigid surface pipes made of metal or PVC (polyvinyl chloride). It is used to convey water to the field, or it can be fitted with outlets or gates to dispense water directly into individual furrows.

Humphreys' invention uses one layflat tube instead of the two previously required by combining conveyance and distribution into a single tube. The key is a flexible inner membrane that can move from side to side within the tube, so water can either pass through the tube or flow from outlets in the side of the tube into the furrows.

The tubing lengths vary from about 100 to 300 feet, which corresponds to an irrigation "set." Both the amount of water available—based on a farmer's water delivery—and the capacity of the irrigation system limit the area that farmers can irrigate during a given time. So they divide their fields into

ALLAN HUMPHERYS

**A membrane running lengthwise in this irrigation tubing shunts water into the furrows or with a simple move of a valve channels it through the tubing to more distant furrows or another field.**

segments, or sets of furrows, that are irrigated one set at a time.

Diverter valves and couplers, made from short sections of metal or PVC pipes, connect individual lengths of tubing.

Humphreys tested the new tubing on 45 acres of furrow-irrigated barley, corn, and alfalfa during the 1989-90 growing seasons. On a farm near Grandview, Idaho, the new system replaced a ditch irrigation system.

"Now, instead of moving and setting 60 siphon tubes many times during an irrigation, the irrigator simply pulls a handle on the manual valves," says Humphreys. The farmer, who had kept the ditch available in case the new system failed, recently filled it in, because the new tubing worked so well, Humphreys noted.

This spring, Humphreys began testing the system on 30 acres of cotton and 30 acres of processing tomatoes in collaboration with ARS researchers Claude J. Phené, James E. Ayars, and Harry I. Nightingale at the Water Management Research Unit in Fresno, California.

There, the researchers will use an even more advanced version of the system. The diverter valves are equipped with electric timers that automatically switch the irrigation sets. Small solar panels, about the size of a sheet of paper, sit atop the valves, providing power to run the timers.

The main advantage, says Phené, is making irrigation more uniform within a field. Changing sets manually can lead to delays, which may result in overwatering. That can increase the likelihood of erosion and leaching of agricultural chemicals.

Back in Colorado, Oest is busy working on ways to mass-produce the tubing for commercial use.—By **Julie Corliss**, ARS.

*Allan S. Humphreys is with the USDA-ARS Soil and Water Management Research Unit, Route 1, 3793 N 3600 E, Kimberly, ID 83341 (208) 423-6514. Claude J. Phené, James E. Ayars, and Harry I. Nightingale are with the USDA-ARS Water Management Research Unit, 2021 S. Peach Ave., Fresno, CA 93727. Phone (209) 453-3122.* ◆

## No More Soggy Sandwiches

Fed up with making jelly sandwiches that are soggy by lunchtime?

ARS scientists have a solution—an edible wrap that goes inside your sandwich to form a moisture barrier between the jelly and the bread.

Preliminary experiments show that the wrap, produced at ARS' Western Regional Research Center in Albany, California, can protect bread for 2 days.

Still, ARS research chemist Dominic W.S. Wong cautions that before the product can be used as an edible wrap in home or commercial kitchens, its all-natural ingredients must be approved for food use.

Those ingredients are lauric acid, from coconut oil, and chitosan, a derivative of chitin, a material in the shells of crab, shrimp, crawfish, and lobster. The film's bland taste won't interfere with the flavor of food products.

When heated, mixed, then spread out in sheets to dry, the lauric acid and chitosan make a flexible, silvery sheet. Wong and former ARS chemist Allen G. Pittman, along with colleague Francois A. Gastineau of France, developed the wrap.

Wong suggests the film might work equally well to protect the freshness of highly perishable produce such as cauliflower or cucumber.

Unlike today's most widely used plastic wraps, made from petrochemicals, the chitosan-based wrap is 100 percent biodegradable. And, although the idea of using lauric acid and chitosan for an edible film isn't new, the Albany team, according to chemist Pittman, is apparently the first to extensively test it for this use.—By **Marcia Wood,** ARS.

*Dominic W.S. Wong is with the USDA-ARS Process Chemistry and Engineering Research Unit, Western Regional Research Center, 800 Buchanan St., Albany, CA 94710. Phone (415) 559-5860.* ◆

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## Grasp at Straws, Irrigators Are Advised

Scattering a little straw in furrows can save water and reduce the soil erosion that occurs when farmers irrigate their crops.

A recent study in Kimberly, Idaho, found that wasteful water runoff from the end of the furrows can be cut nearly in half, simply by scattering 1-3 pounds of straw per 100 feet of furrow.

"The straw forms mini-dams that slow the water down, so it backs up and becomes deeper in the furrows," says Melvin J. Brown, a soil scientist who worked on the study. "Soil on the sides of the furrows is porous, so the deeper water soaks in more quickly, right towards the plant's roots."

That extra water for the roots can boost yields—as much as 62 percent in dry beans, according to Brown. He's gotten similar results in fields of sugar beets, corn, and potatoes grown in southern Idaho.

The region's silty loam soils, which are fine and powdery when dry, erode easily during irrigation.

"We've documented large reductions in soil erosion by using straw," says Brown. The results are most pronounced on fields with slopes of 2 percent or greater. These fields are more prone to erosion, so improvements are more apparent.

"Farmers can use straw selectively in areas of their fields that normally don't get enough water—like steep sections and the end of the furrow," says W. Doral Kemper, an ARS soil specialist based in Beltville, Maryland.

More uniform water distribution also helps prevent nitrate fertilizer leaching caused by overirrigation, adds Kemper, who was formerly with the Kimberly research unit.

Several private companies have developed machines that deposit straw in furrows at desired rates, which should speed adoption of the practice, Brown notes.

Recognizing the value of straw mulching, as it is called, USDA's Agricultural Stabilization and Conservation Service (ASCS), with technical assistance from another USDA arm, the Soil Conservation Service (SCS), offers a cost-sharing program for farmers who want to try the technique.

"Farmers in Canyon County, Idaho, for instance, can receive from $30 to $35 per acre with this program," says James Schmollinger, at the SCS office in Caldwell, Idaho. So far, those taking advantage of the program have seen real benefits in saving both soil and water. Note, however, that the amount of government support varies from county to county.—By **Julie Corliss,** ARS

*Melvin J. Brown is with the USDA-ARS Water Management Research Unit, 3793N, 3600E, Kimberly, ID 83341. Phone (208) 423-5582.* ◆

## Off to Market: A Stressful Affair

Going to market may take a greater toll on hogs than previously thought. Typically, it is assumed that hogs headed for market will lose 3 percent of their weight because of travel stresses and deprivation of food and water before shipping.

But ARS researchers found the loss may be greater, depending on how long the hogs are off feed and water and how far they're transported. Going without feed and water for up to 48 hours caused most of the live weight losses—as much as 7 percent—and more than half of the carcass weight losses.

Transport and related activities caused additional weight losses of up to 3 percent. Scientists say the study's findings provide useful information to all parties involved in the shipment of slaughter-weight hogs.—By **Marcie Gerrietts,** ARS.

*For more information, contact G. LeRoy Hahn or John A. Nienaber, USDA-ARS Roman L. Hruska U.S. Meat Animal Research Center, P.O. Box 166, Clay Center, NE 68933. Phone (402) 762-4100.* ◆

## Blocking Insect Immune Response

Like humans, insects can fend off disease-causing organisms that might otherwise kill them. But exactly how these enduring organisms wage an immune defense has been uncertain.

Now, researchers have revealed the specific biochemical messages that trigger an immune response in the tobacco hornworm *(Manduca sexta).* Blocking these messages, they've discovered, weakens the insect's response.

"That leaves the hornworms vulnerable to deadly bacterial infections," says Ralph W. Howard, an ARS chemist who worked on the study.

Researchers might someday be able to use this information to snuff out pests—by using bacteria that's harmless to humans but fatal to bugs with weakened immune responses, he says. Howard is at ARS' Biological Research Laboratory in Manhattan, Kansas.

They injected hornworms with eicosanoid inhibitors, substances that block the production of certain hormones. In higher animals, eicosanoids control many physiological reactions, including immune defenses. Little is known about their role in insects and other invertebrates.

Next, scientists infected the insects with a red-pigmented strain of the bacterium *Serratia marcescens*. These naturally occurring bacteria are typically found on insects and in soil.

By clipping the tip off a hornworm's foreleg, the scientists could collect a few drops of its "blood"—a clear, greenish fluid called hemolymph. The samples were then grown on plates to count the bacteria present.

Hornworms that received inhibitors contained much more bacteria than the control group (insects whose immune systems were not blocked). The higher the dose of the inhibitors, the more bacteria were present. And that led to a higher death rate in the treated hornworms.

When eicosanoid production was blocked, the message telling the insect to wage a cellular-based defense was, in effect, silenced. The cellular immune response includes cells that engulf and destroy bacteria cells, a process known as phagocytosis.

"We knew about the infection and defense processes, but we didn't know what happened in between," says Stanley-Samuelson. "Now we've identified the biochemical pathways that mediate insect immune defense, and they're similar to those in humans. The next step is to find specific inhibitors that affect insects only."

Using this information to control pests is many years away, says Howard. But he speculates the inhibitors could be added to a bait that insects eat, which would damage their immune systems.

"Then we could hit them with a bacterial pathogen," he says. The compromised bugs couldn't withstand infections and would eventually die.

"It's an indirect approach, but these days we're looking for more sophisticated methods than just pouring on a chemical."—By **Julie Corliss**, ARS.

*Ralph W. Howard is at the USDA-ARS Biological Research Unit, U.S. Grain Marketing Research Laboratory, Room 115, 1515 College Ave., Manhattan, KS 66502. Phone (913) 776-2706.* ◆

## Milk Doesn't Block Cereal's Iron

Go ahead and drown your breakfast cereal with milk. It won't interfere with your body's ability to absorb iron from the cereal or other whole-grain foods such as brown rice, says ARS nutrition researcher Judith R. Turnlund.

Results of her study with eight healthy young women at the ARS Western Human Nutrition Research Center in San Francisco indicate that milk neither boosts nor blocks iron absorption. The findings should allay a concern, raised by earlier studies conducted elsewhere, that milk might inhibit the absorption of iron from foods eaten at the same meal.

"In our test," says Turnlund, "milk made no significant difference in the amount of iron our volunteers absorbed from their food."

Cereal products they ate included english muffins and whole-wheat hot cereal at breakfast, a brown rice casserole with stir-fried vegetables at lunch, and chapate, a spicy, tortilla-like bread, at dinner.

Turnlund's findings should be especially good news for women who are trying to boost their intake of milk or other calcium-rich dairy products to prevent osteoporosis. "They can go for the calcium, needed for strong bones," she says, "without worrying that they'll short themselves of the iron they need for good health."

Because they rely heavily on cereal grains for iron, vegetarians might also be heartened by the results. The study's outcome should also encourage people who are eating less red meat than before. "Any nutritional glitch that interferes with iron absorption will affect these people more than others," she explains. "We have shown that milk is not a glitch."

Why is iron absorption a special concern to these two groups of people? "If you eat little or no red meat, you have to be extremely careful to get enough usable iron in your food to avoid iron-deficiency anemia," explains Turnlund. "The form of iron from grains and vegetables, non-heme, is much more difficult to absorb than the heme iron in red meats. Because of this problem, we wanted to monitor milk's effect on cereal's iron."

To track volunteers' absorption of iron, Turnlund spiked the cereal component of each meal with a natural but rare form of iron that's easily traced. That iron, if not absorbed by the body, showed up in stool samples analyzed by a high-tech procedure called thermal ionization mass spectrometry.

The National Dairy Council sponsored the experiment.—By **Marcia Wood**, ARS.

*Judith R. Turnlund is with the USDA-ARS Western Human Nutrition Research Center, Micronutrients Research Unit, P.O. Box 29997, San Francisco, CA 94129. Phone (415) 556-5662.* ◆

# AGNOTES

## Altered Biocontrol Proves Effective Against Damping-Off Disease

A beneficial biocontrol fungus that controls damping-off diseases has been altered to resist a common chemical fungicide, so farmers may one day use the two side by side to control a range of soilborne crop diseases.

ARS research associate Nina Ossanna, under the supervision of geneticist Sue Mischke, genetically altered the fungus *Gliocladium virens* to resist the fungicide benomyl. Both scientists are at the Biocontrol of Plant Diseases Laboratory in Beltsville, Maryland.

In its natural, unaltered form, *G. virens* was recently approved by the Environmental Protection Agency as a greenhouse biocontrol for damping-off diseases of flowering crops.

The diseases also attack cotton, beans, carrots, and other crops, costing more than $1 billion a year. In tests, the natural *G. virens* reduces plant loss to damping-off by 80-95 percent—similar to control offered by chemical fungicides.

Meanwhile, benomyl offers excellent control of anthracnose, fusarium wilt, leafspot, powdery mildew, and other fungal diseases. And it is one of the fungicides still permitted by the Environmental Protection Agency.

But growers couldn't even hope to use the two simultaneously to control the range of soilborne diseases victimizing their crops—at least not until now. The problem, Mischke says, was that "benomyl wipes out *G. virens* entirely under normal circumstances."

So working at the ARS Biocontrol of Plant Diseases Laboratory in Beltsville, Maryland, the researchers inserted into *G. virens* a gene from another fungus that had been mutated with ultraviolet light to resist benomyl.

The result: at least one *G. virens* strain that grows well in the presence of benomyl and that inhibits damping-off organisms in a petri dish.

That means that genetically engineering the fungus didn't destroy its biocontrol ability.

Currently, Mischke is conducting growth chamber tests with the new *G. virens*, damping-off disease organisms, and cucumbers to see how well the new strain protects a crop from damping-off. She applies benomyl and the *G. virens* to soil containing damping-off disease and then plants the cucumbers.

Field trials—and eventual use by farmers—may be years away, because Mischke must first obtain approval from the USDA's Animal and Plant Health Inspection Service and the Environmental Protection Agency to put a genetically engineered organism into the field.

Scientists at Mischke's laboratory had previously used ultraviolet irradiation to develop biotypes of beneficial molds. But this is the first time a biocontrol fungus has been genetically engineered with a practical trait—not just a test trait that proves the organism can be engineered.—By **Jessica Silva,** ARS.

*Sue Mischke is at the USDA-ARS Biocontrol of Plant Diseases Laboratory, BARC-West, Bldg. 011A, Beltsville, MD 20705. Phone (301) 344-4003.* ◆

SCOTT BAUER

**Fungus being filtered out of this molasses solution by geneticist Sue Mischke will be mixed into soil for a biological disease control for plants. (K-4168-5)**

## Letters

Dear Editor:

Your recent feature on the gypsy moth brought to mind the misfortune that befell two beautiful American beech trees next door. They were victimized by that cruel intruder, but in a decidedly roundabout fashion.

Dead as a doornail now, their fates were sealed in the fall of 1989. My fastidious neighbor John Manack, alert to the encroaching horde of the gypsy moths hard by our area of northern Virginia, had put gunnysacks around all his big trees, as per instructions.

But despite vigilance, he found nary a specimen that fall, and in fact had to wait until this summer to discover one on his property.

Two things happened that fall. We had the coldest December on record. And nobody told John to remove the gunnysack. They froze, girdling the trees. The two beeches never leafed out again.

A leaflet on controlling gypsy moths does mention the need to remove the encircling materials in the fall, but not why.

Now all astute readers of *Agricultural Research* know why, thanks to John's revealing if costly and inadvertent demonstration.

Ben Blankenship
Stafford County, Virginia

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